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Improved Toluidine Blue-DNA Agar for Detection of DNA Hydrolysis by Campylobacters

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Our improved toluidine blue-DNA agar was compared with methyl green DNase test agar for the detection of DNA hydrolysis by campylobacters. The toluidine blue-DNA agar gave clear-cut positive and negative reactions with campylobacter strains belonging to several species.

The production of nucleases by various bacteria (2, 3, 6, 8, 9) has been demonstrated by growing the organisms on DNA-containing agar media followed by flooding the plates with hydrochloric acid (2) or DNA-containing media using indicators such as methyl green (MG) (7) and toluidine blue O (3, 6, 8, 9).

DNA hydrolysis by campylobacters was described previously (1, 5) and constitutes one of the criteria of Lior's biotyping scheme for *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter laridis* (4) in which a modified MG-DNase agar (MG-DNA) medium was used for its detection. The test required incubation of the plates for 3 to 5 days, and in many instances positive reactions (colorless, clear zones around the bacterial growth) were difficult to read and interpret.

To improve the interpretation of the test and shorten the incubation period, we have evaluated the toluidine blue-DNA agar (TB-DNA) described by Lachica et al. (3) and present an improved medium which allows clear-cut positive and negative reactions. The TB-DNA medium was prepared by adding to 1 liter of Tris buffer (0.05 M; pH 9.0) (made according to manufacturer's instructions from TRIZMA-9.0; Sigma Chemical Co., St. Louis, Mo.) 0.3 g of DNA (Difco Laboratories, Detroit, Mich.), 1.0 ml of 0.01 M $CaCl_2$, 10.0 g of NaCl, and 6.5 g of agar L28 (Oxoid Ltd., London, United Kingdom). The mixture was heated to boiling for about 25 min or until all the DNA and agar was completely dissolved, after which the medium was cooled to 50°C and 2.5 ml of 3% toluidine blue O (certified stain; Fisher Scientific Co., Pittsburgh, Pa.) in distilled water was added, mixed well, and dispensed into flasks for storage or poured in 25-ml aliquots into petri plates (90 mm). The final pH was about 8.7. The medium did not require sterilization and could be stored in bottles for at least 3 months at room temperature.

We have evaluated the MG-DNA prepared as previously described (4) and the TB-DNA using 523 campylobacter strains: 272 *C. jejuni* strains, 195 *C. coli* strains, 6 *C. laridis* strains, and 50 *C. pylori* strains.

A loopful of a 24- or 48-h culture grown at 36°C was used to inoculate heavily a circular area of about 5 mm in diameter in duplicate on both media, and the plates were examined after 1, 2, 3, and 5 days of incubation at 36 and 43°C under microaerophilic conditions. Positive reactions (clear, colorless zones around the inoculum) required 3 to 5 days of incubation for the MG-DNA media, whereas large, pinkish, clear zones were observed on the TB-DNA after 24 to 48 h of incubation. The best results were obtained when the TB-DNA plates were incubated for 48 h at 43°C. The results obtained with both media are shown in Table 1. All the strains were tested three times on both media without variations in results (data not shown).

TABLE 1. DNA hydrolysis by *Campylobacter* spp.

Species	MG-DNA[a] (no. of results/no. of strains tested)		TB-DNA[b] (no. of results/no. of strains tested)	
	Positive	Negative	Positive	Negative
C. jejuni	130/272	142/272	137/272	135/272
C. coli	41/195	154/195	57/195	138/195
C. laridis	2/6	4/6	2/6	4/6
C. pylori	0/50	0/50	50/50	0/50

[a] Reactions observed after 3 to 5 days of incubation.
[b] Reactions observed after 48 h of incubation.

We recommend TB-DNA as a replacement for the MG-DNA used in Lior's biotyping scheme for campylobacters.

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